CD98 induces LFA-1-mediated cell adhesion in lymphoid cells via activation of Rap1

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Abstract CD98 is a multifunctional heterodimeric membrane protein involved in the regulation of cell adhesion as well as amino acid transport. We show that CD98 cross-linking persistently activates Rap1 GTPase in a LFA-1-dependent manner and induces LFA-1/ICAM-1-mediated cell adhesion in lymphocytes. Specific phosphatidylinositol-3-kinase (PI3K) inhibitors suppressed both LFA-1 activation and Rap1GTP generation, and abrogation of Rap1GTP by retroviral overexpression of a specific Rap1 GTPase activating protein, SPA-1, totally inhibited the LFA-1/ICAM-1-mediated cell adhesion. These results suggest that CD98 cross-linking activates LFA-1 via the PI3K signaling pathway and induces accumulation of Rap1GTP in a LFA-1-dependent manner, which in turn mediates the cytoskeleton-dependent cell adhesion process.

Key words: CD98; LFA-1; Rap1; Phosphatidylinositol-3-kinase; Cell adhesion

1. Introduction

CD98 is a heterodimeric membrane protein consisting of a 85 kDa glycosylated type II protein (H chain) and a 37 kDa multi-spun transmembrane protein with amino acid transport activity (L chain) [1–4]. There are multiple related 'CD98 L chains' covalently linked to a common H chain depending on tissues [5,6]. We have indicated that the H chain functions as a 'chaperon' for targeting the L chain to the plasma membrane [4].

Several studies, on the other hand, have indicated that CD98 is also involved in the regulation of cell adhesion [7,8]. It was reported that anti-CD98 H chain antibody induced cell aggregation and syncytium formation associated with virus infection, which was mediated by α3β1 integrin [7]. More recently, it was shown that the transfection of CD98 H chain cDNA could reverse the dominant negative effect of the chimeric cytoplasmic domain of β1 integrin and activate endogenous β1 integrin [8]. These results have suggested that CD98 is involved in the regulation of integrin functions, although underlying molecular mechanisms remain largely unknown.

Rap1 is a Ras-related small GTPase that is activated by a variety of distinct extracellular stimuli [9]. We have reported that Rap1 plays important roles in the regulation of integrin-mediated cell adhesion [10,11]. In the present study, we show that cross-linking of CD98 increases the avidity of β2-integrin LFA-1 for ICAM-1 and induces the LFA-1-mediated cell adhesion, in which a phosphatidylinositol-3-kinase (PI3K)-dependent activation of Rap1 plays crucial roles.

2. Materials and methods

2.1. Cells and culture

Normal mouse spleen cells were stimulated with Con A for 2 days followed by the culture with IL 2 for 3 days without Con A. BAF3 (pro-B cells) and BAF3 transfected with human LFA-1 α (αL) and β2 chain cDNAs (BAF3/hLFA-1) [11] were cultured in the RPMI1640 medium supplemented with 5×10^{-5} M 2-mercaptoethanol, 10% fetal calf serum and 10% WEHI-3 conditioned medium.

2.2. Antibodies

The $F(ab')_2$ fragment of a monoclonal antibody (moAb) for mouse CD98 H chain, 14.37, was used for the stimulation of cells. Other antibodies used were as follows; anti-human αL (TS1/22), anti-human β2 chain (7E4, Immunotech Inc.), anti-mouse αL (KBA-1, FD441.1), anti-mouse ICAM-1 (KAT-1), anti-mouse VCAM-1 (PS/2), anti-FLAG (Sigma), anti-Rap1 (Transduction Laboratory), anti-phospho-external signal-regulated kinase (anti-phospho-ERK) (Santa Cruz Biotechnology), anti-phospho-AKT (New England Biolabs) and anti-SPA-1 [10].

2.3. Retroviral transfection

BAF/hLFA-1 cells were infected with the retrovirus containing Flag-tagged Spa-1 cDNA [10] in the presence of 10 μg/ml polybrene for 2 h and cultured in the medium supplemented with G418 for 2 weeks.

2.4. ICAM-1 adhesion assay

Cells were labeled with 2′, 7′-bis-(2-carboxyethyl)-5 carboxyfluorescein (Molecular Probes) and transferred into the microtiter wells precoated with purified ICAM-1 (2 μg/well). After 30 min incubation at 37°C, non-adherent cells were removed by needle aspiration, and adherent cells were determined using a fluorescence concentration analyzer (Idexx Corp., USA) [11].

2.5. Immunoblotting, immunostaining and flowcytometry

To detect Rap1 GTP, cells were lysed with lysis buffer (150 mM NaCl, 50 mM Tris–HCl pH 7.6, 0.5% Triton X-100, protease inhibitors), and precipitated with the GST-RalGDS Rap1-binding domain fusion protein conjugated with glutathione-bead at 0°C for 2 h, fol-

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Abbreviations: moAb, monoclonal antibody; PI3K, phosphatidylinositol-3-kinase; ERK, external signal-regulated kinase; NHIg, normal hamster IgG; APC, antigen-presenting cells

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lowed by the blotting with anti-Rap1 antibody. [10]. Immunostaining and flowcytometric analysis was done as before [4].

3. Results

3.1. Anti-CD98 H chain antibody induces cellular aggregation mediated by LFA-1 and ICAM-1

Stimulation of the primary T cell blasts with a $F(ab')_2$ fragment of anti-CD98 H chain moAb (14.37) induced strong cellular aggregation, which was significantly inhibited by anti-LFA-1 or ICAM-1 moAbs (Fig. 1A). To confirm the primary involvement of LFA-1, we employed BAF3 line transfected with human (h) LFA-1, BAF/hLFA-1. Parental BAF3 only marginally expressed LFA-1 with a high level of ICAM-1, and was barely aggregated with the moAb (Fig. 1B, a). In contrast, BAF/hLFA-1, which highly expressed hLFA-1, exhibited strong cellular aggregation (Fig. 1B, b). The same effect was observed in independent BAF/hLFA-1 clones, the degree of aggregation being correlated with the hLFA-1 expression levels (data not shown). As shown in Fig. 1C, aggregation of BAF/hLFA-1 with the moAb was completely inhibited by the 'blocking' anti-hLFA-1 (Fig. 1B, b) as well as anti-mICAM1 (Fig. 1B, c), while anti-mVLA4 was without effect (Fig. 1B, d). Immunostaining analysis revealed that LFA-1 was indeed concentrated in the cell adhesion sites following the stimulation with the moAb, whereas significant proportion of LFA-1 was detected in the perinuclear region in addition to the cell surface in the unstimulated BAF/hLFA-1 (Fig. 1D). These results have indicated that the cross-linking of CD98 induces cellular aggregation mediated by the LFA-1 and ICAM-1 interaction in lymphocytes.

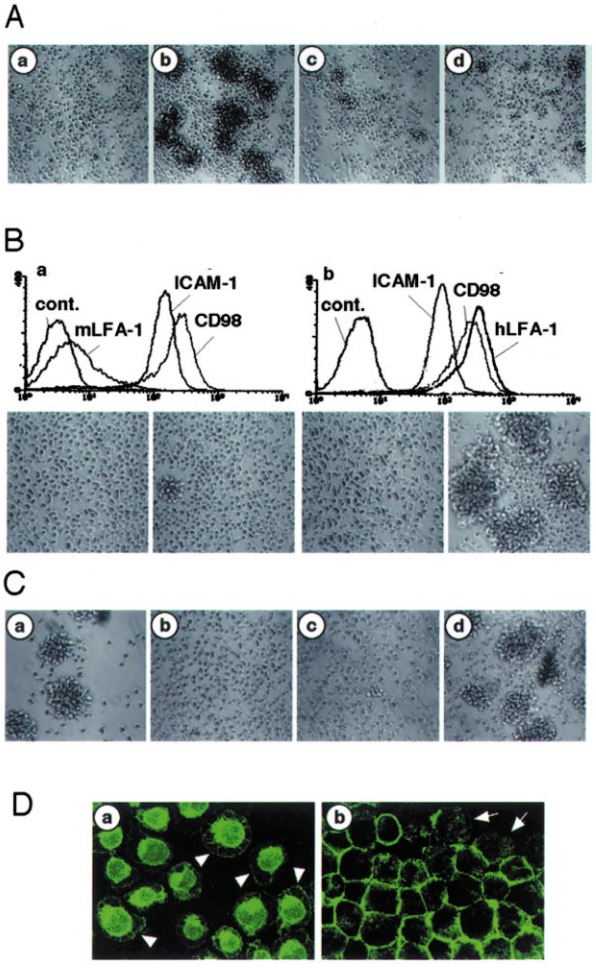

Fig. 1. CD98-induced cell aggregation is mediated by the LFA-1/ICAM-1 interaction. A: The primary T cell blasts were incubated with normal hamster IgG (NHIg) (a) or 14.37 moAb (b–d) at 5 μg/ml for 4 h in the absence (b) or presence of anti-mouse LFA-1, FD441.1 (c) or anti-ICAM-1 (d) moAb at 40 μg/ml. B: Expression profiles of LFA-1, ICAM-1, and CD98 in BAF3 (a) and BAF/hLFA-1 (b). The cells were cultured in the presence of either NHIg (left) or 14.37 moAb (right). C: BAF/hLFA-1 cells were pretreated with medium (a), anti-human LFA-1 (b), anti-mouse ICAM-1 (c) or anti-VCAM-1 (d) moAbs (each at 40 μg/ml) for 30 min followed by the incubation with 14.37 moAb at 5 μg/ml for 4 h. D: BAF/hLFA-1 cells incubated with NHIg (a) or 14.37 moAb (b) for 4 h were cytospined without disturbing the aggregates and immunostained with FITC anti-hLFA-1. Arrowheads indicate the cell surface (left), and arrows the free cell surface (right).

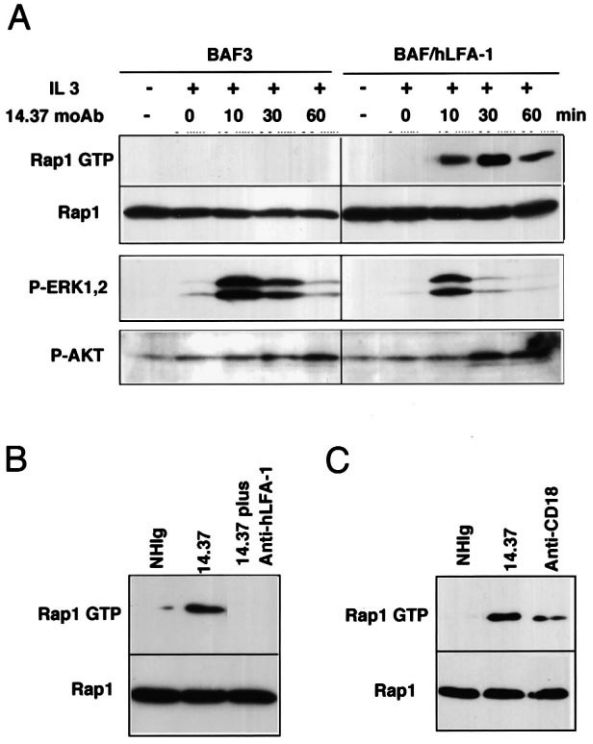

Fig. 2. Anti-CD98 moAb activates Rap1 in a LFA-1-dependent manner. A: BAF3 and BAF/hLFA-1 were incubated at 37°C in the absence or presence of WEHI-CM (IL 3). 14.37 moAb was included in the culture for the indicated periods. Rap1 GTP and total Rap1 were detected as described in Section 2. Aliquots of the samples were examined for the phosphorylated ERK and AKT. B: BAF/hLFA-1 cells were preincubated with or without anti-LFA-1, TS1/22 (40 μg/ml), for 20 min followed by the addition of NHIg or 14.37 moAb (10 μg/ml). Rap1 GTP was detected after an additional 30 min incubation. C: BAF/hLFA-1 cells were incubated at 37°C in the presence of NHIg, 14.37, or anti-CD18 (10 μg/ml each) for 30 min, and Rap1GTP was detected.

3.2. CD98 cross-linking activates Rap1 GTPase in a LFA-1-dependent manner

We have reported that exogenous expression of dominant active Rap1, RapV12, results in the strong cellular aggregation of BAF/hLFA-1 [11]. This prompted us to examine the possible role of endogenous Rap1 in the CD98-induced cell

adhesion. As shown in Fig. 2A, 14.37 moAb induced the generation of Rap1 GTP in BAF/hLFA-1, which persisted for at least 60 min, whereas no significant Rap1 GTP was detected in BAF3. On the other hand, the moAb induced the transient ERK phosphorylation in both BAF3 and BAF/hLFA-1 with an earlier peak than Rap1GTP (within 10 min). In fact, the degree of ERK phosphorylation was significantly less in BAF/hLFA-1 than in BAF3 (Fig. 2A). CD98 cross-linking also induced phosphorylation of AKT in both cells (Fig. 2A). As shown in Fig. 3B, a 'blocking' moAb for hLFA-1 completely inhibited the generation of Rap1GTP by 14.37 moAb in BAF/hLFA-1, while an 'activating' moAb for β2 chain (CD18) alone induced significant Rap1 activation albeit less than 14.37 moAb (Fig. 2C).

3.3. LFA-1-mediated cell aggregation via CD98 cross-linking is dependent on Rap1GTP

We then addressed whether activation of the endogenous Rap1 was indeed involved in the cell aggregation process by CD98 cross-linking. BAF/hLFA-1 over-expressing a specific Rap1 GTPase activating protein, SPA-1 [12], was prepared by retroviral infection (Fig. 3A). BAF/hLFA-1/SPA-1 exhibited no detectable generation of Rap1GTP following the 14.37 moAb stimulation, while those infected with an empty retrovirus (LXSN) showed an increase in Rap1GTP (Fig. 3C). As shown in Fig. 3B, BAF/hLFA-1/SPA-1 showed significantly reduced activation of LFA-1 for ICAM-1 binding by 14.37 moAb as compared with the control (net increase, 8.0% vs. 22.3%). Furthermore, cellular aggregation by the moAb was almost completely suppressed in BAF/hLFA-1/SPA-1 (Fig. 3D). These results have indicated that LFA-1-mediated aggregation via CD98 stimulation depends on the endogenous generation of Rap1GTP, which additionally contributes to the LFA-1 activation per se.

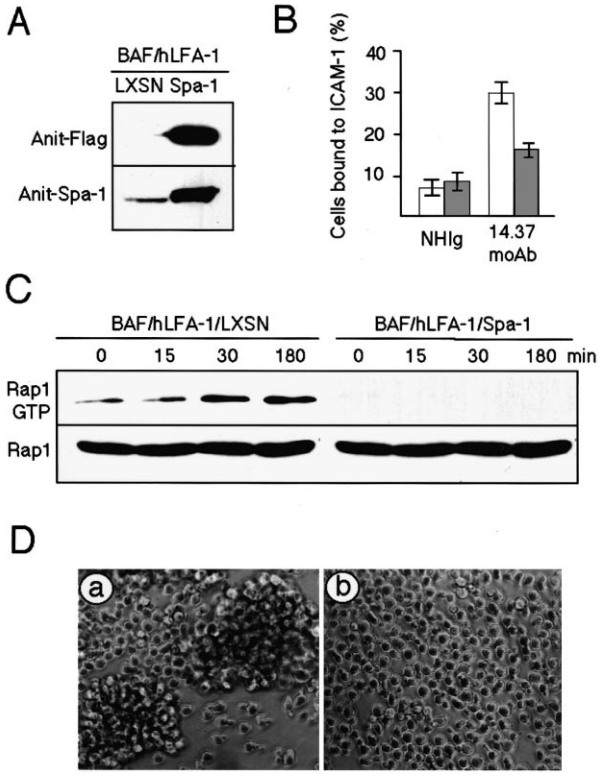

Fig. 3. Retroviral overexpression of SPA-1 abrogates the cell aggregation via CD98. A: BAF/hLFA-1 cells were infected with retrovirus containing Flag-tagged Spa-1 cDNA and the expression of SPA-1 was detected by immunoblotting. B: Fluorescence-labeled BAF/hLFA-1/LXSN (open columns) and BAF/hLFA-1/Spa-1 (filled columns) cells were plated on the ICAM-1-coated wells in the presence of NHIg or 14.37 moAb for 30 min, and the specific adhesion was determined. C: BAF/hLFA-1/LXSN and BAF/hLFA-1/Spa-1 cells were incubated with 14.37 moAb for the indicated periods, lysed, and Rap1GTP as well as total Rap1 were detected. D: BAF/hLFA-1/LXSN (a) and BAF/hLFA-1/Spa-1 (b) cells were incubated with 5 μg/ml 14.37 moAb for 4 h.

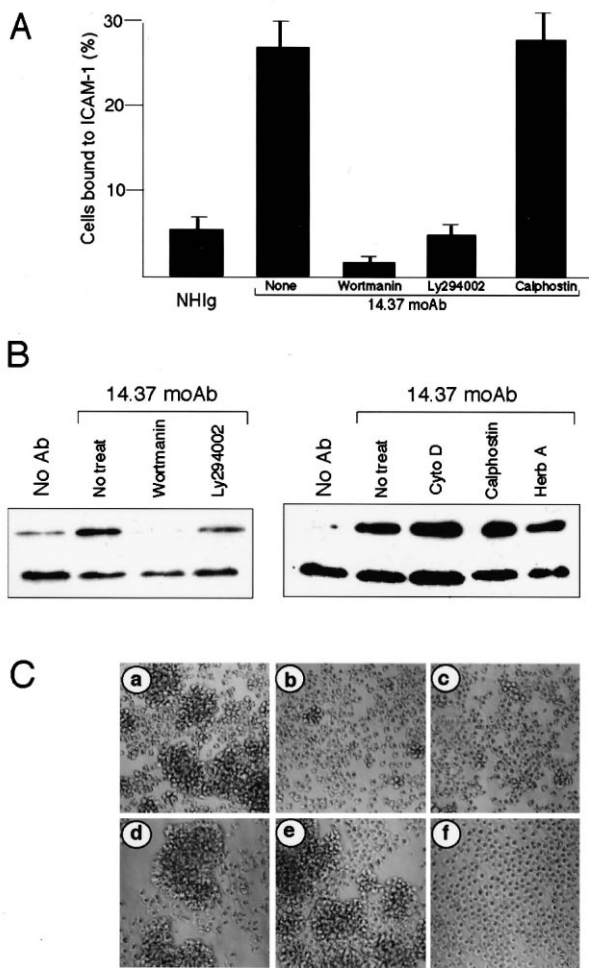

Fig. 4. PI3K inhibitors suppress LFA-1 and Rap1 activation and cell aggregation via CD98. A: Fluorescence-labeled BAF/hLFA-1 cells were pre-incubated for 15 min with medium, wortmannin (50 nM), Ly294002 (50 nM), or calphostin (250 nM), plated on the ICAM-1-coated wells in the presence of 14.37 moAb for 30 min, and adhered cells were quantitated. B: BAF/hLFA-1 cells were pretreated as above with medium, wortmannin, Ly294002, calphostin, herbimycin A (10 μM) or cytochalasin D (2 μg/ml), and then incubated with 14.37 moAb for 30 min. Rap1GTP (upper lanes) and total Rap1 (lower lanes) were detected. C: BAF/hLFA-1 cells were similarly preincubated with medium (a), wortmannin (b), Ly294002 (c), calphostin (d), herbimycin A (e), or cytochalasin D (f) followed by the incubation with 14.37 moAb for 4 h.

3.4. PI3K inhibitors suppress the LFA-1 activation, Rap1 activation, and cell aggregation via CD98

We finally sought the molecular links between CD98 and

LFA-1 activation. As shown in Fig. 4A, a PI3K inhibitor, wortmannin, completely abrogated the adhesion of BAF/hLFA-1 to ICAM-1 in the presence of 14.37 moAb, and another PI3K inhibitor, Ly294002, suppressed the adhesion to the control level. Neither PKC inhibitor calphostin (Fig. 4A) nor PTK inhibitor herbimycin A (data not shown) affected the LFA-1 activation. Conforming to the effects on activation of LFA-1, wortmannin totally suppressed the generation of Rap1 GTP by 14.37 moAb and Ly294002 did so nearly to the control level, while calphostin and herbimycin A were without effect (Fig. 4B). Both PI3K, but not other, inhibitors strongly suppressed the cell aggregation by the moAb (Fig. 4C). Cytochalasin D also inhibited the cell aggregation via CD98, while it hardly affected the Rap1 activation per se (Fig. 4B), indicating the requirement of cytoskeletal reorganization in the former but not the latter process. These results have suggested strongly that a PI3K-mediated signaling is involved in the initiation of LFA-1 activation and Rap1GTP generation via CD98, which is compatible with the increased AKT phosphorylation by the moAb (see Fig. 2A).

4. Discussion

LFA-1 is involved in a number of important cellular interactions including those between leukocytes and endothelial cells for cell migration, T lymphocytes and antigen-presenting cells (APC) for the initiation of immune response, and cytotoxic lymphocytes and target cells [13–15]. In resting leukocytes, LFA-1 is mostly in the 'inactive' state with low ligand affinity, and is activated to the high affinity state by various stimulation, which appears to be associated with conformational changes of the extracellular domain [16,17]. Following the activation, LFA-1 is redistributed on the plasma membrane and clustered to form stable cell–cell adhesion in a cytoskeleton-dependent manner [16,17].

Present study has indicated that the cross-linking of CD98 induces not only activation of LFA-1 for ICAM-1 binding but also cellular aggregation mediated by the LFA-1/ICAM-1 interaction. Cross-linking of CD98 induced rather persistent activation of Rap1 in BAF/hLFA-1 but not in parental BAF3, while it induced ERK activation in both cell types. Thus, Rap1 activation via CD98 was dependent on LFA-1. Abrogation of Rap1 GTP accumulation by exogenous overexpression of SPA-1 totally suppressed the cell aggregation by the moAb. The results clearly indicated that the endogenous Rap1GTP is a crucial mediator for the LFA-1-mediated cell adhesion via CD98. Most recently, it has been reported that the CD31-induced T cell adhesion via LFA-1 and VLA-4 to ICAM-1 and VCAM-1 respectively is also mediated by the activation of Rap1 [18].

Several distinct signaling pathways can mediate the 'inside-out' signaling for integrin activation, including PKC, PI3K, and small GTPases [19–21]. Present results revealed that the activation of LFA-1 via CD98 was completely inhibited by specific PI3K inhibitors, strongly suggesting the involvement of the PI3K signaling pathway. Furthermore, they profoundly inhibited the generation of Rap1GTP and subsequent cell aggregation as well. It was indicated that expression of dominant active PI3K p110 subunit induced LFA-1 activation, although it failed to cause the LFA-1-mediated adhesion [11]. Exogenous RapV12, on the other hand, induced both LFA-1 activation and cell adhesion [11]. Indeed, over-expression of SPA-1 significantly if not completely inhibited the activation of LFA-1 via CD98 as well. We therefore propose that Rap1GTP generated at the down-stream of LFA-1 activated via the PI3K pathway not only mediates the subsequent cytoskeleton-dependent cell adhesion process but also further amplifies the LFA-1 adhesiveness per se in the cells stimulated through CD98, thereby forming a positive feedback loop. This may explain the persistent and progressive cell adhesion in the presence of anti-CD98 moAb.

At present, possible ligands for CD98 remain to be determined, but it was reported that macrophage-derived galectin 3, an efficient molecular cross-linker, could bind to CD98 on T cells [22]. CD98 plays significant roles in the interaction between T cells and APC [23], in which LFA-1 is critically involved [13]. It has been also reported that Rap1GTP is one of the critical factors to determine the T cell responsiveness [24]. Thus, present results may provide a clue to understand the roles this classical antigen CD98 plays.

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